

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
23 February 2006 (23.02.2006)

PCT

(10) International Publication Number
WO 2006/018425 A2

(51) International Patent Classification:

B01D 69/14 (2006.01) **A61B 5/00** (2006.01)

B01D 71/54 (2006.01) **C12Q 1/00** (2006.01)

(21) International Application Number:

PCT/EP2005/054000

(22) International Filing Date: 15 August 2005 (15.08.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

PA 2004 01234 16 August 2004 (16.08.2004) DK

PA 2004 01235 16 August 2004 (16.08.2004) DK

(71) Applicant (for all designated States except US): **NOVO NORDISK A/S** [DK/DK]; Novo Allé, DK-2880 Bagsværd (DK).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **ROSSMEISL, Charlotte C.** [DK/DK]; Flintemarken 55, 2. tv., DK-2950 Vedbæk (DK). **SABRA, Mads Christian** [DK/DK]; Nørrebrogade 36, 4., DK-2200 København N (DK). **GERSTENBERG, Michael** [DK/DK]; Ørbækgårds Allé 608, DK-2970 Hørsholm (DK). **GLEJBØL, Kristian** [DK/DK]; Kvædehaven 109, DK-2600 Glostrup (DK). **BUCH-RASMUSSEN, Thomas** [DK/DK]; Dalvej 28, DK-2820 Gentofte (DK).

(74) Common Representative: **NOVO NORDISK A/S**; Corporate Patents, Novo Allé, DK-2880 Bagsværd (DK).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: MULTIPHASE BIOCOMPATIBLE SEMI-PERMEABLE MEMBRANE FOR BIOSENSORS

(57) Abstract: This invention relates to a novel two phase biocompatible semi-permeable membrane for biosensors and more particularly to a homogenous membrane for use in in-vivo sensing of glucose by an enzyme. The membrane comprises of a continuous hydrophilic phase of water swellable polyurethane having discrete particles of polydimethyl siloxane dispersed therein. Furthermore, a membrane having good properties for use as the outer membrane in a biosensor continuous determination of the blood glucose level in vivo, can be prepared by spraying of a polymer solution in a big number of spraying steps, for example, more than 20 steps.



WO 2006/018425 A2

Multiphase biocompatible semi-permeable membrane for biosensors

This invention relates to a group of novel multi phase biocompatible semi-permeable materials useful for production of biosensors and more particularly of outer membranes suitable for implantable biosensors for in-vivo sensing of glucose. Furthermore, this invention relates to a method for preparing such membranes.

Background of this Invention

In diabetes mellitus, the pancreas loses its ability to manufacture and secrete insulin leading to metabolic imbalance. A result of this condition is that the body loses the ability to regulate the glucose content of the blood. Historically, diabetes mellitus has been treated by insulin injections, diet, exercise and oral medication.

To provide patients with means to improve their metabolic regulation, there is a need to develop a device which is able to continuously monitor the glucose content of the blood. Such a device will have to include one or more biosensors to be implanted in the patient.

A biosensor is an analytical device incorporating a combination of a specific biological element (cells, enzymes, tissues, etc.) and a physical element that transduces the recognition event into a detectable signal (electrical, acoustic, optical, thermal etc). Typically, the sensor will produce a signal that is quantitatively related to the concentration of the analyte. There are many types of biosensors used for a wide variety of analytes. Electrochemical bio-sensors typically use enzymes to convert a concentration to an electrical signal. Immunological biosensors rely on molecular recognition of an analyte, for example, antibodies, (cf. Principles of Chemical and Biological Sensors, Chemical Analysis vol. 150, John Wiley & Sons, Inc., 1998; Biosensors in the Body, D. M. Fraser, John Wiley & Sons, 1997).

Regardless of the type of biosensor, each material that constitutes the biosensor must possess certain properties to function in vivo and provide an adequate signal. First, the outermost surface of the biosensor in contact with tissue must be biocompatible, i.e. perform with an appropriate host response in a specific application (Williams, "A model for biocompatibility and its evaluation", J. Biomed. Eng., 185-191, **11** (1989)). Biocompatibility includes not causing a direct injury or only causing a minor or inferior injury to the living tissue, any adverse response, other adverse systemic effects or delayed adverse effects (Wallin, "Global biocompatibility", Med. Dev. Tech, 34-38, **6** (1995)). To be of any practical importance, it is furthermore important that the biosensor signal is stable and not adversely affected by the presence of proteins as well as electrolytes, medications and other potentially interfering compounds. To prevent interference from proteins, the outer membrane of a sensor should suppress protein adhesion. Furthermore, the sensor should employ one or more layers keeping potentially interfering compounds away from the active parts of the sensor.

Some biosensors are depending on more than one chemical species to function. One prominent example on this is amperometric glucose sensors employing oxido-reductase enzymes which uses oxygen as a co-substrate (see Fig. 2). If more chemical species are required for the proper function of the sensor, it is vital that the species (analyte) of interest limits the output of the sensor rather than the required co-substrate. For glucose sensors employing oxido-reductase enzymes, it is thus important that excess oxygen (relative to glucose) is present in order to give valid readings. This is in the following referred to as the "oxygen deficient problem". Although the typical glucose concentration in the tissue of a diabetic patient is about 40-100 times higher than the oxygen concentration, the "oxygen deficient problem" can be solved by choosing a membrane material which permeability to oxygen is higher than that of glucose. To summarize, a successful membrane for a glucose sensor employing oxido-reductase enzymes have to be biocompatible, have to shield towards interfering chemical species, and have to be more permeable to oxygen than to glucose.

In the literature, numerous examples are given on membrane systems suitable for glucose employing oxido-reductase enzymes.

US Patent No. 4,759,828 discloses a micro porous membrane for addressing the "oxygen deficit problem". Micro porous membranes may work well during short term experiments. However, fouling of the membrane material due to protein adhesion might influence the long-term stability. Furthermore, due to the porosity, the electrodes and the enzyme layer of the sensor are exposed to body fluids containing proteins. The proteins degrade the sensor performance rapidly due to fouling of the electrodes and deterioration of the active enzyme.

US Patent No. 4,484,987 uses a combination membrane with discrete domains of a hydrophobic material embedded in a hydrophilic membrane. Although simple to describe and test in the laboratory, working membranes having stable long-term stability, sufficient permeability, as well as adequate mechanical strength have proven extremely difficult to produce due to the formation of large structures showing poor cohesion.

US Patent Nos. 5,882, 494 and 5,777,060 describe a homogenous polymer composition, which is a reaction product of at least one diisocyanate, at least one hydrophilic diol or diamine, and at least one silicone material. The porous membrane in this patent incorporates two different types of bonds and components into a single polymer. The reaction of a diisocyanate and a diol makes the urethane linkage and the same isocyanate reacts with diamine to make a urea linkage. Hence, in these patents, siloxane is a part of the reaction product.

Production of coatings and membranes from a solution is a well established technology widely known in the art. Very simple approaches like dipping or painting often result in coatings having sufficient quality. But if complex geometries are to be coated or if special demands are put on the application process, spray coating may be a preferred strategy. In US patent No. 2,378,148 is given the layout for a spray coating system special in that the coating material has to be heated prior to application. Heating as a mean to enhance the spray process is also applied in US patent No. 4,505,957.

One major obstacle with the production of thick membranes according to the teaching of US Patent No. 4,484,987, i.e., membranes containing hydrophobic domains, is that the cohesion between the hydrophobic and the hydrophilic phase is poor. Thus, if the membrane is produced from a solution, the resulting

membrane will be inhomogeneous and possibly porous. The inhomogeneity is a result from the fact that the hydrophobic molecules form domains during evaporation of the membrane solution. These domains may act like nucleation centers for voids formed due to relaxation of the membrane.

5 In US Patent No. 5,882,494 is disclosed a method according to which hydrophobic/hydrophilic block co-polymers are utilized in the production of membranes. Due to the immobilization of the hydrophobic blocks to the hydrophilic back-bone of the polymer-chains, this method ensures a homogenous dispersion of small hydrophobic domains in the hydrophilic matrix. Although the use of a
10 block co-polymer ensures a homogenous membrane, the use of a single block co-polymer system is in the general case not beneficial as one loses the possibility of tailoring a specific polymer-system to a specific application. Additionally, the polymer described in US Patent No. 5,882,494 requires relatively thin membranes due to the relatively limited transportation of glucose in this polymer system. Due to the thin membrane, it is required that the sensor is protected from
15 exposure directly to the tissue. Finally, experiments have shown that this type of membranes are susceptible to protein adhesion and thus to decreasing sensitivity during use.

Although simple to describe and test in the laboratory, working membranes having stable long-term stability, sufficient permeability as well as adequate mechanical strength have proven extremely difficult to produce.
20

Micro porous membranes may work well during short term experiments. Due to the porosity, the electrodes and the enzyme layer of the sensor are, however, exposed to body fluids containing proteins that degrade the sensor performance rapidly due to fouling of the electrodes and deterioration of the active
25 enzyme.

The only identified way to prevent deterioration of the sensor is to use an apparently homogenous and dense membrane, thus preventing the proteins in reaching the inner parts of the sensor. Polyurethane is known as a suitable material due to the documented bio-compatible properties of this material (G. W.
30 Shaw, D.J. Claremont and J.C. Pickup, Biosensors and Bioelectronics, 401-406, **6** (1991); D. S. Bindra, Y. Zhang, G. S. Wilson, R. Sternberg, D. R. Thevenot, D. Moatti and G. Reach, Analytical Chemistry, 1692, **63** (1991); and M. Shichiri, Y.

Yamasaki, K. Nao, M. Sekiya and N. Ueda, Horm. Metab. Res., Suppl. Ser., 17, **20** (1988)). Although fulfilling the demands for a stable and mechanically strong sensor, sensors covered with a dense membrane of, for example, polyurethane are not suitable as glucose sensors due to the oxygen deficit problem discussed earlier in this text.

From the literature, it was thus concluded that a membrane having sufficient strength, longevity and oxygen permeation could be produced based on polyurethane (PU) mixed with polysiloxane (sil), denoted PU/sil, using the principle mentioned in US patent No. 4,484,987 (see Fig. 3).

As mentioned in the text above, porous and otherwise defective membranes are not feasible for long term use. Also, very thin membranes should be avoided as they by nature are extremely fragile. Although solutions for handling thin fragile membranes are found in the literature (see, for example, "An electroenzymatic glucose sensor fabricated on a flexible substrate", J. J. Mastrototaro *et al.*, Sensors and Actuators B, 139-144, **5** (1999)) this is clearly not a viable way if a sensor is to be made as small and efficient as possible.

To allow for enhanced diffusion of glucose, advantage is taken from the scheme suggested by Allen in US patent. No. 5,322,063 wherein a polyurethane (PU) system modified with polyethyleneoxide (PEO) is used to enhance the diffusion of glucose as well as oxygen.

Experiments inspired by the approach suggested by Allen proved that a PU/PEO copolymer based system with sufficient permeability towards glucose can be made. The experiments did, however, also show that it is not possible to produce a viable sensor according to this scheme due to the fact that glucose as well as oxygen diffuses through the same phase, resulting in that the permeability of glucose relative to oxygen is fixed and that the permeability towards glucose is too high relative to the permeability of oxygen.

Surprisingly, the experiments using PU/PEO copolymer showed additional and unexpected advantages. Due to the PEO content, the PU/PEO copolymer absorbs water (swells). As the swelled polymer transports water very efficient to the inner parts of the sensor this dramatically decreases the start-up time of the system. An additional benefit of the swelling polymer system is that tensile stresses induced in the membrane during production is partly or fully balanced

by the volume expansion, thus resulting in a membrane system which in the use situation is free of internal stresses.

Further descriptions of glucose biosensors can be found in "In vivo characteristics of Needle Type Glucose Measurements of subcutaneous glucose concentrations in Human Volunteers", Shinchri et al, *Horm. Metab. Res., Suppl Ser.*, 17-20, **20** (1988); "In vivo measurements of subcutaneous glucose concentrations with an enzymatic glucose sensor and a wick method", Bruckel *et al.*, *Klin. Wochenschr.*, 491-495, **67** (1989); "In vivo molecular sensing in diabetes mellitus: an implantable glucose sensor with direct electron transfer", Pickup *et al.*, *Diabetologia*, 213-217, **32** (1989); "Biosensors in the Body", Fraser, John Wiley & Sons, 1997; "Implanted Electrochemical Glucose Sensors for the Management of Diabetes", Heller, *Annu. Rev. Biomed. Eng.*, 153-175 (1999); "A new amperometric glucose microsensor: In vitro and short term in vivo evaluation", Ward *et al.*, *Biosensors and Bioelectronics*, 181-189, **17** (2002); "Materials and Techniques for Electrochemical biosensor design and construction", S. Zhang et al., *Biosensors and Bioelectronics*, 273-282, **15**, (2000); "An electroenzymatic glucose sensor fabricated on a flexible substrate", J. J. Mastrototaro *et al.*, *Sensors and Actuators B*, 139-144, **5** (1999); "Enzyme-Based Biosensors for *in Vivo* Measurements", G. S. Wilson and Y. Hu, *Chem. Rev.* 2693-2704, **100** (2000); "A Subcutaneous Glucose Sensor with Improved Longevity, Dynamic Range, and Stability of Calibration", Updike *et al.*, *Diabetes Care*, 208-214, **23** (2000); "A Continuous Glucose Sensor Based on Wired Enzymed™ Technology – Results from a 3-Day Trial in Patients with Type 1 Diabetes", Feldman *et al.*, *Diabetes Technology & Therapeutics*, 769-779, **5** (2003); all of which are hereby incorporated by reference.

Objects of this Invention

It is an object of this invention to devise a multiphase material suitable as the outer membrane of glucose sensors based on an enzyme using oxygen as a co-substrate. The invention is in particular suitable for transdermal sensors employing one or more oxido-reductase enzymes.

Yet another objective is to ensure high mechanical strength of the membrane and thus ensure mechanical integrity of the system in realistic use situations.

Yet another object of this invention is to provide a membrane having bio-compatible properties.

Furthermore, it is the object of this invention to devise a novel method which enables the production of isotropic membranes from a solution containing multiple immiscible polymers, for example, a membrane of this invention. This object of the invention is accomplished by applying the membrane in many steps, each step comprising application of diluted membrane material and subsequent drying. By applying the membrane in many consecutive steps, diffusion and phase-separation is prevented, thus yielding a virtually homogenous membrane.

Yet another objective is to enable production of multi-phase membranes containing both hydrophobic oxygen transporting domains as well as hydrophilic domains enhancing the biocompatibility of the system, for example, a membrane of this invention.

Yet another object of the present invention is to provide a membrane having good long-term stability during *in vivo* use, for example, a membrane of this invention.

Summary of this Invention

Briefly, a main object of this invention is accomplished by enhancing the permeability of oxygen of a membrane by inclusion of domains made from a polymer having high permeability towards oxygen but low permeability towards the species oxidized in the sensor into another polymer or a mixture of miscible polymers already permeable to oxygen and immiscible with the first polymer.

To achieve some of the aforementioned objects, i.e. in relation to the membrane, this invention comprises a membrane system for a biosensor peculiar in that the membrane consists of a mixture of at least two immiscible polymers as defined more closely in claim 1 below. One of the immiscible polymers is preferably a

PU/PEO or PU/polytetramethylene glycol (PTMG) copolymer and, preferably, the other immiscible polymer is enhancing oxygen permeation.

The membrane of this invention combines several essential properties for amperometric glucose sensors, namely a high sensitivity (high signal-to-noise ratio), large linearity range, high chemical and mechanical stability necessary for in-vivo use, and biocompatibility.

Preferably, the immiscible oxygen enhancing polymer forming the discrete particles in the membrane is either a liquid or a solid having a molecular weight above 10 kDa.

Furthermore, to achieve other of the aforementioned objects, i.e. in relation to the method of this invention, this invention comprises a method for application of a membrane system for a biosensor, peculiar in that the membrane is made from an isotropic compound consisting of at least two immiscible polymers, for example, a membrane of this invention.

To prevent precipitation of domains having a size comparable with the thickness of the membrane, the membrane is made in many consecutive steps, each step consisting of application of material and subsequent evaporation of solvent. By evaporating of solvent between the application steps, precipitation of larger domains is prevented, thus resulting in a virtually isotropic membrane. In one embodiment of this invention, the membrane is prepared by more than 30 consecutive application/evaporation steps, preferably more than 100 steps, even more preferred more than 500 steps.

To facilitate a homogenous mixing of the polymers before application, at least one of these is dissolved. The other immiscible polymers are either dissolved with the first immiscible polymer or suspended in the solution as particles or both.

In this context, a solvent is either a pure compound as, for example, ether or a mixture of compounds like alcohol and water, characterized in that the solvent is able to dissolve at least one of the immiscible polymers and that, preferably, the final polymer solution has a viscosity below about 50 cSt at 25°C, more preferred below about 20 cSt at 25°C.

Possible although not necessary, the layers of the membrane might vary, for example, such that some of layers first applied to the substrate show low permeability towards glucose whereas some of the layers later or last applied to the substrate show high permeability towards glucose.

5

Definitions

Herein, the "amperometric oxido-reductase enzyme based biosensor" used for the tests of the product of this invention means a two-electrode electrochemical sensor (as depicted in Fig. 1) comprising a working electrode with largest cross sectional dimensions of $350\mu\text{m} \times 40\mu\text{m}$ made from a paste of platinum particles, and a reference electrode with largest cross sectional dimensions of $300\mu\text{m} \times 40\mu\text{m}$ made from a Ag/AgCl polymer paste. The length of the electrode which is intended for implant into the skin is about 15 mm, whereas the uncovered working electrode is about 2.4 mm and the uncovered reference electrode is about 11 mm. The electrodes are made by screen-printing the pastes, which are custom made for the purpose. The electrodes are printed on opposite sides of a polyimide substrate with cross-sectional dimensions of approximate $500\mu\text{m} \times 180\mu\text{m}$. The working electrode is covered by an inner membrane of a mixture of cellulose triacetate and Nafion[®] with a thickness of 1 to 8 μm . On top of the inner membrane, an enzyme layer of thickness 1 to 8 μm made from glucose oxidase and glutaraldehyde is deposited. A dielectric paste for insulate the conductor tracks and limit the electrode area and contact pads. The product described herein, and denoted an outer membrane, is applied as a complete cover over the electrodes, substrate, dielectric paste and other membranes. The sensor is configured as a dipole running at a constant voltage of 0.6V. The sensor defined above is also equivalent to an "electrochemical glucose sensor".

Herein, the "linearity range for measurements of glucose" can be obtained by calculating the currents corresponding to the different glucose concentrations (1, 2.00, 2.99, 4.98, 6.95, 9.90, 14.8, 19.6, 24.4, 29.1, 33.8, and 38.5 mM glucose) by taking a five minute average, 10 minutes after the glucose addition. Both average and standard deviation should be calculated. Expected outliers within the ranges are tested with Dixon's Q test ("Statistics for Analytical Chem-

istry", Miller and Miller, 3rd edition, Ellis Horwood Ltd., 1993) at significance level 5%. The linear range (current = sensitivity x glucose concentration + background) by fitting the first three data with linear regression. If none of the points deviate more than 10% (relative error) from the linear fit, continue by adding the next point. Points are added until the relative deviation between fit and current values are larger than 10%. A linear fit is performed for these points. If all points are within 10% error for the new fit, the current values for the next concentrations are tested similarly. The linear range is defined as the range within which all points deviates relatively less than 10% for the linear regression fit.

Herein, for 'signal-to-noise ratio', the noise is defined as the fluctuations of the current of an amperometric oxido-reductase enzyme based biosensor where a constant background has been subtracted from the average current. Time-varying contributions to the current of non-glucose origin, such as interferents (for example, ascorbic acid, acetaminophen, and uric acid), is not included in the background, but they are expected to give negligible contributions to the measured currents.

Herein, the term "extended linearity range for measurements of glucose" means that the current response to glucose addition is linear in a range 5 mM broader, when comparing the linearity range of an amperometric oxido-reductase enzyme based biosensor having an outer membrane of the test material with the linearity range of an amperometric oxido-reductase enzyme based biosensor having an outer membrane of a material identical with the test material with the proviso that it does not contain the high oxygen permeability material. This test is performed in a stirred (Magnetic stirrer, IKA® color squid magnetic stirrer, stirring speed setting: 10 o'clock) standard PBS buffer (pH 7.4, 150 mM NaCl) at reduced oxygen tension ($pO_2=60$ mm Hg) at a temperature of 37°C.

Herein, the term "ultimate tensile strength in wet condition" is determined by an elongation of a polymer thin film having approximate dimensions of 10 mm wide x 20 mm long x ~30 μ m thick at 30 mm/min until rupture using a Lloyds instruments tensile rig LR5K with a 20 Newton measuring head. The polymer has been immersed for at least 6 hours in PBS buffer (pH 7.4, 150 mM

NaCl) prior to the tensile test, which is performed within 5 minutes after lifting the thin film from the buffer.

Herein, the term "a sufficient biocompatibility in vivo" means that the device performs with an appropriate host response in a specific application (Williams, 1995) thus ensuring a stable biosensor response when implanted subcutaneously in, for example, the arm or the abdomen.

Herein, the term "permeability towards oxygen" or oxygen permeability means the transmission of oxygen molecules through a polymer film. A definition of the permeability P is $P = (\text{quantity of permeant, here oxygen}) \times \text{film thickness} / (\text{film area} \times \text{time} \times \text{pressure or concentration drop across the film})$. The quantity of the permeant is typically expressed by mass, moles, or gaseous volume at standard temperature (273.15K) and pressure (1.013×10^5 Pa) (STP). The quantities given here are given in volume at STP. For experimental details, see Stern and Bhide, J. Appl. Polymer Sci., 2131, **38** (1989); Stern *et al.*, J. Polym. Sci. B, 1263, **25** (1987).

Herein, the term "without changing the properties in an undesired way" means that an amperometric oxido-reductase enzyme based biosensor used for the tests of the product according to this invention retains its linearity range with respect to glucose, response time, stability and other properties before and after, for example, e-beam irradiation.

Herein, the term "a sufficient low start up time" means that an amperometric oxido-reductase enzyme based biosensor used for the tests of the product of this invention reaches its equilibrium condition after immersion in a glucose containing fluid within 1 hour.

Herein, the term "stable response of a biosensor" (or stable biosensor response), means that a glucose sensor, such as the one defined above as an amperometric oxido-reductase enzyme based biosensor, inserted 3 days in the subcutaneous tissue has a detectable glucose signal, i.e. a signal-to-noise ratio larger than 3 and a sensitivity larger than 0.4 nA/mM/mm^2 , with a signal decay of less than 20% per day. The insertion of the sensor in the subcutaneous tissue (approximately 2 mm below the skin surface) is performed by a needle like insertion device which can penetrate the skin without damaging the sensor and be

removed after use. The subcutaneous tissue in the abdomen is typically used as measuring site, but other sites can also be utilized such as the upper arm.

Herein, the term "long term stability" means that a sensor, as described in Fig. 1 and exemplified above in the definition of "an amperometric oxido-
5 reductase enzyme based biosensor" and which has an outer membrane in accordance with this invention, has a current, when measured in-vitro in a PBS buffer (pH 7.4, 150 mM sodium chloride (NaCl)) at reduced oxygen tension (30-60 mm Hg), linear up to at least 20 mM glucose giving a sensitivity of at least 0.4
10 nA/mM/mm² at given times after production of the membranes (up to several months, for example, 6 months, or even years, for example 2 years). In addition, the signal does not decrease more than 20% per day for 3 days when the sensor is measured at a given times after manufacture.

Herein, the term "good chemical stability" means that a sensor such as the above defined amperometric oxido-reductase enzyme based biosensor with
15 an outer membrane of this invention has both the herein defined stable response and the herein defined long term stability.

Herein, the term "good mechanical stability" means that an outer membrane of this invention when applied on a sensor exemplified in the definition of an amperometric oxido-reductase enzyme based biosensor does not delaminate
20 or does not rupture after 3 days insertion in the subcutaneous tissue. The invention covers ranges for an outer membrane which yield strength and ultimate elongation which are, for example, measured by the procedure mentioned in Example 6.

Herein, the term "polyurethane" refers to a polymer containing at least
25 two urethane linkages.

Description of the Accompanying Drawings

Fig. 1 illustrates an amperometric glucose sensor employing an oxido-reductase
30 enzyme designed for subcutaneous or intravenous use.

Fig. 2 illustrates the reactions taking place in the enzyme containing layer of the sensor, below the outer membrane.

Fig. 3 illustrates a membrane as described by Fig. 2 of US Patent No. 4,484,987.

Fig. 4 illustrates an enlarged scale illustration of the membrane according to this invention.

Fig. 5a & 5b shows the in-vivo (pig) sensor signal for a pure polyurethane membrane (Thermedics HP60D-20) and with 18.6 wt % polydimethylsiloxane (DC360) dispersed therein. The addition of the polydimethylsiloxane clearly enhances the sensor response to approx. 20 mM (Fig. 5b) from merely 10 mM (Fig. 5a).

Fig. 6 shows ESEM image of an outer membrane on a glucose sensor (18.6 wt % DC360 in Thermedics HP60D-20, fully hydrated).

Fig. 7a is a scanning electron microscope image of a cross section (perpendicular to the outer surface) of a membrane of this invention spray deposited onto a flat substrate (not visible in this figure). The white inclusions (domains) represent the hydrophobic silicone domains (PDMS 12500 cSt) in a continuous matrix of hydrophilic polyurethane.

Fig 7b is a close up of the membrane in Fig. 7a where the silicone domains have been colored dark.

Fig. 8: ATR-FTIR measurements of a membrane (18.6 wt % DC360 in Thermedics HP60D-20): Silicone and polyurethane identified in final processed membrane.

Fig. 9 shows the current response of an amperometric biosensor to glucose in an PBS buffer having an outer membrane of this invention.

Fig. 10 shows DSC measurement of a membrane of this invention.

Fig. 11 shows a comparison of discrete blood glucose measurements (Hemocue) and the response of a subcutaneously implanted amperometric glucose sensor with an outer membrane as described in this invention and exemplified in Example 1 for day 2 and 3 after insertion. One calibration was performed 24 hours after insertion.

Fig. 12. Example of a tensile test of a thin polymer film

Fig. 13 illustrates a peeled off membrane from polyurethane and polydimethylsiloxane (hereinafter designated PDMS) prepared according to the procedure suggested in U.S. Patent No. 4,484,987. Note that large voids are present

in the interface between the membrane and the substrate. It is believed that these voids are formed due to cohesion failure of the weak PDMS phase during evaporation of the solvent.

Fig. 14 is a surface of membrane similar to the one illustrated in fig. 3 made from polyurethane and PDMS. Note that large number of visible voids and crevices. It is believed that these are formed due to cohesion failure of the weak PDMS phase during evaporation of the solvent.

Detailed description of this invention

This invention relates to a membrane comprising a continuous phase of one polymer (or a mixture of miscible polymers) and discrete domains of a second high molecular weight polymer with high oxygen permeability (permeability towards oxygen), where the polymers in each phase are immiscible, and where the second high molecular weight polymer has a domain size in the range from about 20 μm to about 1 nm, preferably from about 10 μm to about 10 nm, more preferred from about 5 μm to about 50 nm, and, when said product is used as a dense or mostly dense outer membrane of a glucose oxidase based biosensor, it results in

- a) a signal-to-noise ratio larger than 3,
 - b) sensitivity larger than 0.4 nA/mM/mm²,
 - c) an extended linearity range for measurements of glucose,
 - d) good chemical stability, and
 - e) good mechanical stability
- during *in-vivo* use for measurements of glucose using said glucose oxidase based biosensor as defined herein.

The term immiscible herein designates that the continuous phase is not or only to a minor degree mixed with the polymer forming discrete domains. In other words, discrete domains of the second polymer are present in the polymer or polymer mixture forming a continuous or substantial continuous phase.

In one embodiment, the membrane of this invention is one wherein one of the two polymers is a hydrophilic polymer.

In one embodiment, the membrane of this invention is one wherein one of the two polymers is a hydrophobic polymer.

5 In one embodiment, the membrane of this invention is one wherein the continuous phase is hydrophilic and the discrete domains are hydrophobic.

In one aspect, the membrane of this invention comprises a continuous phase of one polymer (or a mixture of miscible polymers) and discrete domains of a second high molecular weight polymer, where the polymers in each phase
10 are immiscible. The polymers can be immiscible through their hydrophobic/hydrophilic character. In one embodiment of the membrane of this invention, the hydrophobic polymer is a polysiloxane, fluoro-carbon polymer or their block-copolymers.

One way of preparing the membrane of this invention is by mixing a liquid polysiloxane with a dissolved polyurethane resulting in a solution which – after spray drying - can easily be applied as outer membrane to sensors. Due to the immiscible nature of polyurethane and polysiloxane, the former being hydrophilic and the latter hydrophobic, one of the materials **6** (See Fig. 4) will precipitate as domains in the other **7** when the solvent evaporates, thus forming the
20 desired multiphase system (see Fig. 4) where the average domain size of the hydrophobic domains **6** preferably is in the range from about 20 μm to about 1 nm, more preferred from about 10 μm to about 10 nm, even more preferred from about 5 μm to about 50 nm.

In one embodiment, the membrane of this invention is one wherein the molecular weight of the hydrophobic polymer is in the range from about 10 kDa to about 100 kDa, preferably from about 20 kDa to about 80 kDa, more preferred from about 30 kDa to about 60 kDa, most preferred about 42 kDa. In one
25 embodiment, the membrane of this invention is one wherein the molecular weight of the hydrophobic polymer is at least about 10 kDa, preferably at least about 20 kDa, more preferred at least about 30 kDa, most preferred about 42 kDa, and preferably not more than about 60 kDa.
30

Fig. 7a shows a scanning electron microscopy image of a membrane of this invention. The small light domains seen in Fig. 7a are the small hydrophobic

domains of polydimethylsiloxane. To highlight the hydrophobic domains (phase 2 in Fig. 7b), these domains have been colored black (dark), in order to make them visible.

In one embodiment, the membrane of this invention is one wherein one of the immiscible polymers is a PU/PEO or PU/polytetramethylene glycol (designated PTMG) copolymer. The membrane system according to this invention may be made from a compound consisting of at least two immiscible polymers where at least one of the polymers is a PU/PEO or PU/PTMG copolymer and the other polymer has a high permeability towards oxygen. In one embodiment, this invention relates to a product as described above which is prepared using a hydrophobic polymer having a permeability towards oxygen in the range from about 7×10^{-12} to about $7 \times 10^{-10} \text{ cm}^3 (273.15\text{K}, 1.013 \times 10^5 \text{ Pa}) \times \text{cm}/(\text{cm}^2 \times \text{s} \times \text{Pa})$, preferably about 1.4×10^{-11} to about $3.5 \times 10^{-10} \text{ cm}^3 (273.15\text{K}, 1.013 \times 10^5 \text{ Pa}) \times \text{cm}/(\text{cm}^2 \times \text{s} \times \text{Pa})$, more preferred about 2.3×10^{-11} to about $2.1 \times 10^{-10} \text{ cm}^3 (273.15\text{K}, 1.013 \times 10^5 \text{ Pa}) \times \text{cm}/(\text{cm}^2 \times \text{s} \times \text{Pa})$, most preferred about $7 \times 10^{-11} \text{ cm}^3 (273.15\text{K}, 1.013 \times 10^5 \text{ Pa}) \times \text{cm}/(\text{cm}^2 \times \text{s} \times \text{Pa})$.

It is from the above description apparent that the novelty underlying this invention is, *inter alia*, a membrane wherein the siloxane is present as discrete domains in the continuous phase of polyurethane without the formation of any co-polymer. The permeability of glucose and oxygen can be adjusted at will and thereby the linearity region of a non-mediated electrochemical glucose sensor by simply altering the concentration of silicone in the polyurethane. In one embodiment, this invention relates to a product as described above wherein the content of hydrophobic material is in the range from about 1 % to about 50 % (weight/weight) of the total weight, preferably from about 5 % to about 25 %, more preferred from about 8 % to about 20 %, most preferred about 18.6 %.

The PU/PEO or PU/PTMG copolymer used according to this invention is in an embodiment a polymer belonging to the family of polyurethanes. As used herein, the term "polyurethane" refers here to a polymer containing at least two urethane linkages. PU/PEO or PU/PTMG copolymers are readily available from commercial sources such as Thermedics, for example, under the name of Tecophilic. In one embodiment, this invention relates to a product as described above wherein the hydrophilic polymer is a water-swellable polyurethane. The polyure-

thane may comprise hydrogels such as polyvinylalcohol, poly(2-hydroxyl-methacrylate) (polyHEMA), polyvinylpyrrolidone (PVP), or polyethyleoxide (PEO). In one embodiment, this invention relates to a product as described above containing at least one hydrophilic polymer.

5 In an embodiment, the membrane of this invention comprises one or more layers. Herein, the invention comprises double layer membranes, where the innermost layer, which in an amperometric biosensor (see Fig. 1) will be closest to electrode, made from PU/PDMS or PU and the outermost layer is made from PU-PEO, PU-PTMG, PU-PEO/PDMS, PU-PTMG/PDMS, PU/PU-PEO/PDMS, or
10 PU/PU-PTMG/PDMS. The invention comprises, furthermore, a triple layer membrane, where the innermost layer consists of PU, PU-PEO, or PU-PTMG, the second layer of PU-PEO/PDMS, PU-PTMG/PDMS, PU/PU-PEO/PDMS, PU/PU-PTMG/PDMS, and the third layer of PU-PEO, or PU-PTMG. Herein, PDMS is polydimethylsiloxane.

15 Typically, polyurethanes are formed by combining diisocyanates with alcohols and/or amines. For example, combining isophorone diisocyanate with PEG 600 and aminopropyl polysiloxane under polymerizing conditions provides a polyurethane/polyurea composition having both urethane (carbamate) linkages and urea linkages. Diisocyanates which are useful in the preparation of biocompatible polyurethanes are described in detail in Szycher (Seminar on advances in
20 medical grade polyurethanes. Technomic Publishing, (1995)) including both aromatic and aliphatic diisocyanates. Examples of suitable aromatic diisocyanates include toluene diisocyanate, 4,4'-diphenylmethane diisocyanate, and 3,3'-dimethyl-4,4'-biphenyldiisocyanate. Suitable aliphatic diisocyanates include, for
25 example 1,6-hexamethylene diisocyanate (HDI), trimethylhexamethylene diisocyanate (TMDI), trans-1,4-cyclohexane diisocyanate (CHDI), 1,4-cyclohexane bis(methylene isocyanate) (H₆XDI), isophorone diisocyanate (IPDI), and 4,4'-methylenebis(cyclohexylisocyanate). A number of these diisocyanates are available from commercial sources such as Aldrich Chemical Company (Milwaukee,
30 Wis, USA) or can be readily prepared by standard synthetic method using literature procedures.

To facilitate a homogenous mixing of the polymers, the PU/PEO or PU/PTMG copolymer is dissolved in a solvent. The other immiscible polymer is

either dissolved with the first immiscible polymer, suspended in the solution as particles, or dissolved in its own solvent which allows a homogenous mixture of the total dispersion. In this context, a solvent is either a pure compound as , for example, ether or a mixture of compounds like alcohol and water, characterized in that the solvent is able to dissolve at least one of the immiscible polymers.

By choosing a polyurethane with a specified water uptake, herein a polyurethane which swells from about 1% to about 50% of its dry resin weight, preferably from about 5 to about 40%, more preferred from about 10 to about 30%, most preferred about 20%, the yield stress and maximum elongation of the membrane can be designed for any given application. In one embodiment, this invention relates to a membrane as described above having an ultimate tensile strength in wet condition in the range from about 0.1 MPa to about 50 MPa, preferably in the range from about 1 MPa to about 40 MPa, more preferred in the range from about 2 MPa to about 30 MPa, most preferred about 8 MPa. The ultimate elongation of the polymer varies from about 150% to about 800% of its original length, preferably between about 200% and about 700%, more preferably from about 250% to about 600%.

Furthermore, the underlying novelty is, *inter alia*, the choice of a silicone with a very high molecular weight and thus viscosity, which limits migration of silicone within the membrane. Polyurethane and silicone are biocompatible materials that ensure a minimal in-vivo response. In one embodiment, this invention relates to a product as described above which is prepared from a hydrophobic material having a viscosity above about 5000 centistokes, preferably above about 10,000 centistokes, more preferably above about 12,000 centistokes.

In one embodiment, the membrane of this invention is one wherein any hydrophilic polymer is a water swellable polyurethane or compositions of block copolymers of polyurethane, for example, polyurethane/polyethylene oxide, polyurethane/polytetramethylene ether glycol, or polyurethane/polydimethylsiloxane.

In one embodiment, the membrane of this invention is one wherein the polyurethane-polysiloxane block copolymer is utilized as a compatibilizer/emulsifier for stabilisation of polysiloxane domains.

In one embodiment, the membrane of this invention is one wherein the hydrophilic polymer is preferably one from the family of aliphatic, polyether based polyurethanes named Tecophilic from Thermedics Inc., more preferably Tecophilic HP60D-20 from Thermedics Inc.

5 In one embodiment, the membrane of this invention is one wherein the hydrophobic polymer is polydimethylsiloxane, preferably DC360 (viscosity 12,500 cSt, Medical grade) from Dow Corning.

In one embodiment, the membrane of this invention is one which is prepared from a hydrophilic polymer which swells from about 1% to about 50% of
10 its dry resin weight, preferably from about 5 to about 40%, more preferred from about 10 to about 30%, most preferred about 20%.

In one embodiment, the membrane of this invention is one containing at least one or more hydrophilic polymers.

In one embodiment, the membrane of this invention is one where the
15 polymer in suspension or solution is in the range from about 0.1% to about 10%, more preferable from about 0.25% to about 2% (weight/weight).

In one embodiment, the membrane of this invention is one where the solvent is either THF, dimethylformamide, an alcohol, or water or a mixture of the mentioned solvents.

20 In one embodiment, the membrane of this invention is one where the solvents and/or solvents include antioxidants (for example, butylated hydroxytoluen (herein designated BHT)).

In one embodiment, the membrane of this invention is one where the content of THF is in the range from about 60% to about 100% (volume/volume),
25 more preferably from about 70 to about 90%.

In one embodiment, the membrane of this invention is one where the solvent for the polydimethylsiloxane is preferably chosen from the group consisting of hexamethyldisiloxane, octamethyltrisiloxane, decamethyltetrasiloxane, or a mixture thereof.

30 In one embodiment, the membrane of this invention is one sufficient biocompatibility *in vivo* as defined herein.

In one embodiment, the membrane of this invention is one wherein the hydrophilic water swellable material is a polyurethane.

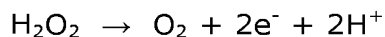
In one embodiment, the membrane of this invention is one wherein the content of hydrophobic material is in the range from about 1 % to about 50 % (weight/weight) of the total weight, preferably from about 5 % to about 25 %, more preferred from about 8 % to about 20 %, most preferred about 18.6 %.

5 In one embodiment, this invention relates to a two or more phase bio-compatible membrane consisting of a membrane of this invention.

Membrane coated biosensors (see Fig. 1), in particular glucose sensors which utilize glucose oxidase to effect a reaction of glucose and oxygen, are known in the art. Fig. 1 shows an example of an amperometric 2-electrode electro-chemical glucose sensor employing an oxido-reductase enzyme designed for sub-cutaneous use. The sensor is comprised of polymer substrate **1**, electrodes **2**, an anti-interference membrane **3**, an enzyme layer **4**, and an outer membrane **5**. The enzyme can either be deposited as a layer (as shown in Fig. 1) or be embedded in the anti-interference membrane. Fig. 2 shows the reactions taking place in the enzyme layer of an amperometric glucose sensor. Due to the presence of the enzyme, the oxidation of β -D-glucose by molecular oxygen is catalysed producing glucono- δ -lactone and hydrogen peroxide via the catalytic reaction:



The gluconolactone further reacts with water to hydrolyze the lactone ring and produce gluconic acid. Thus, one molecule of oxygen is consumed and one molecule of hydrogen peroxide is produced for each glucose molecule. The electro-chemical method detects the changes in either oxygen or hydrogen peroxide relating the measured signal to the concentration of glucose. Hydrogen peroxide (H_2O_2) reacts electrochemically on a electrode surface with an applied potential of 0.6 V as shown below



30 Provided that excess oxygen is present, the rate of this reaction is proportional to the concentration of glucose. The ultimate current is, therefore, proportional to the amount of glucose that reacts with the enzyme. Fig. 9 shows the current

from a glucose sensor immersed in PBS buffer (pH 7.4, 150 mM NaCl) at reduced oxygen tension (30-60 mm Hg), where the outer membrane of the sensor is produced according to this invention. The response to glucose addition is linear, thus showing no sign of "the oxygen deficit problem". Furthermore, Fig. 5a and 5b shows the continuous sensor current measured *in vivo* (pig) and the corresponding blood plasma values. Fig. 5a has no silicone dispersed in the polyurethane resulting in a response only linear to approximately 10 mM, whereas the addition of 18.6 wt % PDMS (DC360, 12500 cSt, Dow Corning) clearly enhances the linearity range to at least 20 mM glucose.

In a further aspect, this invention relates to an implantable biosensor having an outer membrane consisting of a product as described above. Hence, this invention also relates to an implantable biosensor for measuring the reaction of an analyte and oxygen, the working electrode of the sensor including a layer of enzyme, characterized in that said biosensor has an outer membrane consisting of a membrane of this invention.

Basically, this invention depends not on the configuration of the biosensor, but rather on the use of the two phase biocompatible membrane of this invention to cover the sensor elements. In one embodiment, this invention relates to a membrane as described above having a thickness in the range from about 1 μm to 60 μm about, preferably from about 5 μm to about 50 μm , more preferred from about 20 μm to about 40 μm , most preferred about 35 μm . An example of a membrane of a spray-deposited membrane on an electrode for a biosensor is shown in Fig. 6, 7a and 7b. In Environmental Scanning Electron Micrograph (see Fig. 6), a cross section of the outer membrane is seen as the dark region on top of the image. The membrane is fully hydrated at 100% RH and is expected to be fully swelled. The membrane is clearly homogeneous and dense. In one embodiment, this invention relates to a membrane as described above having a volume in the range from about $0.01 \times 10^{-10} \text{ m}^3$ to about $250 \times 10^{-10} \text{ m}^3$, preferably from about $0.5 \times 10^{-10} \text{ m}^3$ to about $150 \times 10^{-10} \text{ m}^3$, more preferred from about 10^{-10} m^3 to about $50 \times 10^{-10} \text{ m}^3$, most preferred about $5 \times 10^{-10} \text{ m}^3$.

In another aspect, this invention relates to an implantable biosensor for measuring the reaction of an analyte and oxygen, the working electrode of the

sensor including a layer of enzyme, said biosensor having an outer membrane consisting of a membrane of this invention. In another embodiment, this invention relates to an implantable biosensor as described above wherein the analyte is glucose. In another embodiment, this invention relates to an implantable biosensor as described above wherein the enzyme is glucose oxidase.

In one embodiment, this invention relates to a product as described above having a sufficient biocompatibility *in vivo*. An implanted glucose sensor with an outer membrane of this invention implanted for 3 days in the subcutaneous tissue (stomach) in a human did not result in any adverse events (allergic response or other immune responses). Furthermore, only a single calibration set (sensitivity, background current, and a time delay between blood and subcutaneous blood values) after day 1 was necessary for achieving a correspondence between the two set of glucose values (see Fig. 11) for day 2 and day 3. Thus, the absence of signal decay after a 3 day period highlights the stability and biocompatibility of the membrane.

In one embodiment, this invention relates to a product as described above which can be e-beam sterilized using a dose 2×25 kGy without changing the properties in an undesired way.

In one embodiment, this invention relates to a membrane which has a long term stability of at least 232 days after production, thus highlighting that no migration of the polysiloxane seems to happen.

In an embodiment, the product does not show any phase transitions in the region from 6°C to 45°C which may alter the properties of the membrane and is thus stable to temperature changes in the given interval as evidenced by the differential scanning calorimetric measurement of a thin film membrane of the described invention seen in Fig. 10.

In another embodiment, this invention relates to an implantable biosensor as described above, having a sufficient low start up time as defined herein.

In one embodiment, the implantable biosensor of this invention is one having a stable biosensor response for 3 days when inserted in the subcutaneous tissue.

In one embodiment, the implantable biosensor of this invention is one comprising a double layer membrane, where the innermost layer, which in an

amperometric biosensor (see Fig. 1) will be closest to the electrode, and is made from PU/PDMS or PU and the outermost layer is made from PU-PEO, PU-PTMG, PU-PEO/PDMS, PU-PTMG/PDMS, PU/PU-PEO/PDMS, or PU/PU-PTMG/PDMS.

In one embodiment, the implantable biosensor of this invention is one comprising a triple layer membrane, where the innermost layer consists of PU, PU-PEO, or PU-PTMG, the second layer of PU-PEO/PDMS, PU-PTMG/PDMS, PU/PU-PEO/PDMS, PU/PU-PTMG/PDMS, and the third layer of PU-PEO, or PU-PTMG.

The method of this invention is a method for placing a layer consisting of two immiscible polymers on a substrate, for example, for preparing a membrane according to any one of the preceding membrane claims, characterized in

- a) using a solvent, preparing a solution or suspension containing one or more polymers,
- b) applying a part of said polymer solution or suspension through a spray nozzle to said substrate in such a way that a certain amount of said solvent is evaporated before the polymer solution/suspension reaches the substrate,
- c) after the remaining part of the polymer solution or suspension has reached the substrate, allowing a substantial part of the remaining amount of solvent present in the polymer solution/suspension to evaporate,
- d) applying a part of said polymer solution or suspension through a spray nozzle to said substrate in such a way that a certain amount of said solvent is evaporated before the polymer solution/suspension reaches the substrate
- e) after the remaining part of the polymer solution or suspension has reached the substrate, allowing a substantial part of the remaining amount of solvent present in the polymer solution/suspension to evaporate, and
- f) repeatedly applying a solution or suspension as described in step b) to said substrate and allowing the solvent to evaporate as described in steps b) and c) for a total of at least about 30 steps, in this order.

In the method of this invention, the solvent used to dissolve the coating material is heated above the boiling temperature of the solvent hereby reducing the need for excessive amounts of solvent.

Numerous examples on schemes for reducing the amount of solvent and hereby making the spray coating more efficient exists in the literature. The present invention is peculiar in that an efficient process, i.e., a process allowing for thick coatings is not wanted. On the other hand, many thin coats are wanted, which allows for some control over the resulting microstructure of the coating.

The method of this invention depends not on the way the coating is applied, but rather on the use of many coating steps consisting of application steps and evaporation steps. In one aspect, the method of this invention relates to a method for placing a layer consisting of at least two immiscible polymers on a substrate. In one embodiment, the method of this invention covers the production of outer membranes for glucose sensors. The preferred substrate includes an electrode formed by screen printing technology or by thin film technology, wherein the substrate optionally is coated with other membrane layers such as enzyme layer or other polymer membranes. The substrate could be polyimide or polyester, whereas the electrode could include platinum.

A solution or suspension containing one or more polymers is applied through a spray nozzle to a substrate in such a way that a certain amount of the solvent present in the polymer solution/suspension is evaporated before the polymer solution/suspension reaches the substrate. After the remaining part of the polymer solution/suspension has reached the substrate, a substantial part of the remaining amount of solvent, present in the polymer solution/suspension, is allowed to evaporate, before a new amount of the polymer solution/suspension is sprayed onto the substrate now with the previous layer of polymer. The time period from the point of time when one amount of polymer solution/suspension reaches the substrate to the point of time where the next amount of polymer solution or suspension reaches the substrate is preferably in the range from about 50 milliseconds to about 10 seconds.

In this way, a polymer solution/suspension is repeatedly sprayed onto the substrate for a total of preferably at least about 30 steps, preferably at least about 100, and more preferred at least about 500. The thickness of the layer de-

posited in each step is preferably below about 5 μm , preferably below about 1 μm and even more preferably below about 100 μm .

In an embodiment of the method of this invention, the amount of solvent, which is evaporated before the polymer solution/suspension reaches the substrate, is in the range from about 80 % to about 99 % (volume/volume). Hence, the content of solvent remaining in the polymer solution/suspension applied in one step is below about 19 % (volume/volume), preferably below about 10 %, more preferred below about 1 %, before a further amount of polymer solution/suspension is applied in the following step.

The method of this invention which is used for application of the polymer solution or suspension may be a particle generating process, for example a spray process, where a nozzle with an air supply is used. Within this embodiment, more than one spray nozzle can be used at the same time, giving the possibility to spray two or more different polymer solutions or suspensions at the same time or at substantially the same time on the same substrate. More than one spray nozzle can also be used sequentially. This could give the advantage of making a lamellar structure if each nozzle sprays different polymer solution or suspensions.

In the method of this invention, the coating is made from a compound consisting of at least two immiscible polymers where at least one of the polymers is soluble. In an embodiment of the method of this invention, the soluble polymer belongs to the family of polyurethanes. As used herein, the term "polyurethane" refers to a polymer containing at least two urethane linkages. PU/PEO (PU is polyurethane and PEO is polyethyleneoxide) copolymers are readily available from commercial sources such as Thermedics, for example, under the name Tecophilic HP-60D-20. Polyurethane/polydimethylsiloxane copolymers are readily available from commercial sources such as The Polymer Technology Group, for example PurSil. In an embodiment of the method of this invention, the amount of polymer in the solution or suspension is in the range from about 0.1 % to about 10 % (weight/weight) and more preferred from about 0.25 % to about 2 % (weight/weight).

In an embodiment, the method of this invention relates to a method wherein the soluble polymers are cellulose triacetate and Nafion in combination.

If one of the polymers is not soluble in the chosen solvent, this has to be suspended in the solution. Although suspension of active particles in dissolved polymers is not special (see, for example, US patent No. 6,355,058), the particles used in the method of this invention are special in that, preferably, their size
5 has to be smaller than about 1/10 of the thickness of the final coating, that is preferable below about 5 μm , and even more preferably below about 1 μm . Among particles, silioxanes and fluoropolymers are preferred due to the high oxygen permeation in these materials. In an embodiment of the method of this invention, polydimethylsiloxane, which is, for example, available from Dow Corn-
10 ing under the name DC360 (Medical grade), with a viscosity of at least 600 cSt and more preferred at least about 12,500 cSt is used.

To solvate the polymers, one or more solvents can be used. Several organic solvents can be used such as tetrahydrofuran, hexamethyldisiloxane, octamethyltrisiloxane, decamethyltetrasiloxane, dimethylformamide, hexan, and
15 heptan alone or in combination. Furthermore, water can be used in combination with one or more organic solvents. In an embodiment of the method of this invention, the solvent is a mixture of tetrahydrofuran, hexamethyldisiloxane and water in the proportions (weight/weight) 60-100% tetrahydrofuran, 0-25% hexamethyldisiloxane, and 0-10% water and more preferred 70-90% tetrahydro-
20 furan, 10-20% hexamethyldisiloxane, and 0-10% water.

In one embodiment, the method of this invention is one wherein one of the polymers is polyurethane or compositions of copolymers of polyurethane, for example, polurethane/polyethyleneoxide or polyurethane/polydimethylsiloxane.

25 In one embodiment, the method of this invention is one wherein one of the polymers is Tecophilic HP-60D-20 from Thermedics.

In one embodiment, the method of this invention is one wherein one of the polymers is Pursil from The Polymer Technology Group.

30 In one embodiment, the method of this invention is one wherein one of the polymers is cellulose triacetate or a composition of cellulose triacetate and Nafion.

In one embodiment, the method of this invention is one wherein the other polymer is selected from the group consisting of polydimethylsiloxane, fluoropolymers, and siloxanes.

5 In one embodiment, the method of this invention is one wherein the polydimethylsiloxane has a viscosity of at least about 600 cSt, more preferred at least about 12,500 cSt.

In one embodiment, the method of this invention is one wherein the polydimethylsiloxane is DC360 (12500cSt, Medical grade) from Dow Corning.

10 In one embodiment, the method of this invention is one wherein more than about 90 %, preferably more than about 95 %, more preferred more than about 99 % of one of the polymers is dissolved in the solvent.

In one embodiment, the method of this invention is one wherein the final layer consists of 3 or 4 different polymers.

15 In one embodiment, the method of this invention is one wherein, in one or more of the steps b), d) etc., a solution or suspension containing 3 different polymers is used.

20 In one embodiment, the method of this invention is one wherein the amount of polymer in the solution or suspension according to step a) is in the range from about 0.1 % to about 10 %, more preferred from about 0.25 % to about 2 % (weight/weight).

In one embodiment, the method of this invention is one wherein the substrate includes an electrode optionally coated with other membrane layers such as enzyme layer or other polymer membranes.

25 In one embodiment, the method of this invention is one wherein the substrate is a polyimide or a polyester.

In one embodiment, the method of this invention the membrane is applied to an electrode formed by screen printing technology or thin film technology.

30 In one embodiment, the method of this invention is one wherein the electrode includes platinum.

In one embodiment, the method of this invention is one wherein the solvent is selected from the group consisting of tetrahydrofuran, hexamethyldisilox-

ane, octamethyltrisiloxane, decamethyltetrasiloxane, dimethylformamide, hexan, heptan, and a mixture of two or more of these solvents.

In one embodiment, the method of this invention is one where the solvent consists of a mixture of water and two ore more organic solvents.

5 In one embodiment, the method of this invention is one where the solvent used is a mixture of tetrahydrofuran, hexamethyldisiloxane, and water.

In one embodiment, the method of this invention is one where the content of tetrahydrofuran is within the range from about 60 % to about 100 % (weight/weight), preferably from about 70 % to about 90 % (weight/weight).

10 In one embodiment, the method of this invention is one where the content of hexamethyldisiloxane is within the range from about 0 % to about 25 % (weight/weight), preferably from about 10 % to about 20 % (weight/weight).

In one embodiment, the method of this invention is one where the content of water in the solvent is within the range from about 0 % to about 10 % (weight/weight).

15 In one embodiment, the method of this invention is one wherein the application of the polymer solution or suspension according to step b), d), etc. is performed by a particle generating process.

20 In one embodiment, the method of this invention is one wherein the application of the solution or suspension according to step b), d), etc. is performed by a spray process using a nozzle with an air supply.

In one embodiment, the method of this invention is one wherein an amount in the range from about 80 % to about 99 % (volume/volume) of the solvent is evaporated before the polymer solution/suspension reaches the substrate (i.e. evaporated in step b), d) etc.).

25 In one embodiment, the method of this invention is one wherein the time period from the start of a step allowing a substantial part of the remaining amount of solvent present in the polymer solution or suspension to evaporate to the preceding step allowing a substantial part of the remaining amount of solvent present in the polymer solution or suspension to evaporate (such as from the start of step b) to the start of step d)) is in the range from about 50 milliseconds to about 10 seconds.

In one embodiment, the method of this invention is one wherein the total number of steps b), c), d), e), etc. is at least about 30, preferably at least about 100, more preferred at least about 500.

5 In one embodiment, the method of this invention is one wherein the polymer solution or suspension applied in steps b), d) etc. has the same or substantially the same composition.

In one embodiment, the method of this invention is one wherein the polymer solution or suspension applied in steps b), d) etc. does not have the same or substantially the same composition.

10 In one embodiment, the method of this invention is one wherein the first layers applied are a primer of one polymer and the next layers are made from another polymer solution.

15 In one embodiment, the method of this invention is one wherein more than one spray nozzle is used at the same time or sequentially with the same or different polymer solutions.

20 In one embodiment, the method of this invention is one wherein the content of solvent in the polymer solution or suspension applied in the last step is below about 19 % (volume/volume), preferably below about 10 %, more preferred below about 1 %, before a further amount of the polymer solution or suspension prepared as described in step a) is applied in the following step.

In one embodiment, the method of this invention is one wherein the layer consists of one phase of polymer wherein a second phase of polymer is dispersed.

25 In one embodiment, the method of this invention is one wherein the dispersed polymer has a domain size below about 5 μm , preferably below about 1 μm .

In one embodiment, the method of this invention is one wherein the thickness of the layer deposited is below about 5 μm , preferably below about 1 μm , even more preferably below about 100 nm.

30 In one embodiment, the method of this invention is one wherein the amount of polymer in the suspension or solution is in the range from about 0.1% to about 10%, more preferable from about 0.25% to about 2% (weight/weight).

In one embodiment, the method of this invention is one wherein the solvent used is either tetrahydrofuran, dimethylformamide, an alcohol, or water or a mixture of the mentioned solvents.

In one embodiment, the method of this invention is one wherein the sol-
5 vents and/or solvents used include antioxidants, for example, butylated hydroxy-
toluen.

In one embodiment, the method of this invention is one wherein the con-
tent of tetrahydrofuran in the solution used is in the range from about 60% to
about 100% (volume/volume), more preferably from about 70 to about 90%.

10 In one embodiment, the method of this invention is one wherein the solvent for
the polydimethylsiloxane is preferably chosen from the group consisting of hexa-
methylidisiloxane, octamethyltrisiloxane, decamethyltetrasiloxane, or a mixture
thereof.

15 Though this invention has been referred to primarily in the determination of glu-
cose concentration in solutions, it is understood that the membrane of this inven-
tion is not limited to the use of this material but may be used for the concentra-
tion determination of other compounds.

20 It will be understood by those skilled in the art that the foregoing general
description and the following detailed description are exemplary and explanatory
of the invention and are not intended to be restrictive thereof. Thus variations
may be made within the scope and spirit of the accompanying claims without
sacrificing the principal advantages of the invention.

25 The mentioning herein of a reference is no admission that it constitutes
prior art.

Herein the word "comprise" is to be interpreted broadly meaning "in-
clude", "contain" or "comprehend" (vide, for example, Guidelines for Examination
in the European Patent Office, 2000, part C, chapter III, 4.13).

All articles referred to herein are hereby incorporated by reference.

30 The following examples are offered by way of illustration and are not meant to
limit the scope of this invention.

Example 1

This example illustrates how to manufacture a membrane of this invention aimed
5 for use in a glucose detecting biosensor by spraying a mixture of a polymer solution onto a substrate resulting in a membrane.

A commercially available polyurethane, which swells 20% compared to dry resin weight, is dissolved in 9.5THF:0.5H₂O. To this, 18.65 weight % (of the total dry weight of polymer) of polydimethylsiloxane dissolved in hexamethyldisiloxane was dispersed in the polyurethane solution to obtain a membrane of this
10 invention.

40 ml milli-Q-water is added to 760 ml tetrahydrofuran giving a total mass of 724g. Then 3.64 g polyurethane, (tecophilic HP-60D-20 purchased from Thermedics) which have been dried in an oven at 55°C for at least 3 hours, are
15 added to the solvents. The 0.5 % tecophilic HP-60D-20 in 9.5THF:0.5H₂O solution is stirred for at least 60 hours.

4.5 g polydimethylsiloxane (DC360, 12500cSt, purchased from Dow Corning) is added to 900 ml hexametyldisiloxane, (OS10 purchased from Dow Corning) giving 0.65 weight % polydimetylsiloxane in hexamethyldisiloxane.

20 128.41 g of 0.65 weight % polydimethylsiloxane in hexamethyldisiloxane is added to 800 ml of 0.5 weight % tecophilic in 9.5THF:0.5H₂O and the solution is stirred until the complete solvation of siloxane (approximately 30 min). The relation between siloxane and total amount of polymer is then calculated to be 18.65 %.

25 The solution is sprayed using a 0.3 mm nozzle with a flow of material of 1.8E-4 m³/h carried by an air flow with a pressure of 2 bar. During spray the object to be coated is mounted on a turntable rotating at 33 RPM thus resulting in an evaporation time of approximately 1.8 seconds between consecutive coats.

The membrane is sprayed for 70 minutes revealing a total thickness of
30 the membrane of around 30 µm. 2310 layers were applied. An ATR-FTIR (attenuated total reflection Fourier Transform infrared) spectrum of the product (see Fig. 8) shows the expected bands from polyurethane and polydimethylsiloxane.

Example 2

This example illustrates the evaluation of a membrane-coated biosensor constructed according to this invention.

A two electrode system (see definition of an amperometric oxidoreductase enzyme based biosensor) where a outer membrane was spray-coated with an approximately 35 μm thick membrane with the composition described in Example 1. The sensor is immersed in a standard PBS buffer (pH 7.4, 150mM NaCl) at reduced oxygen tension (30-60mm Hg) and subsequent the immersion an initial pulse given of 1.1 Volt for 360 seconds is applied. The sensor is kept at 0.6 V for the remaining measurements. After waiting additional 60-70 minutes, the sensor has reached a constant background, and glucose is added at concentrations of 1, 2.00, 2.99, 4.98, 6.95, 9.90, 14.8, 19.6, 24.4, 29.1, 33.8, and 38.5 mM to the solution to measure the linearity of the response. After each addition of glucose, the solution is left to equilibrate for 10 minutes. The solution was continuously stirred (Magnetic stirrer, IKA® color squid magnetic stirrer, stirring speed setting: 10 o'clock). A typical sensor response is shown in the graph depicted by Fig. 9. The sensor response was linear between 1 and 40mM, which clearly shows that the sensor response is not limited in oxygen concentration. Membranes without the polysiloxane addition to the polyurethane show a saturation of the signal at lower concentrations.

Example 3

This example illustrates how a membrane consisting of polyurethane and silicone can be applied on an electrode by dip coating.

Prior to dipping in the membrane solution, the electrode is dipped in a surface enhancer to optimize the uniformity of the finished membrane. 15 wt% Triton X-100 (Sigma-Aldrich) dissolved in ethanol is used as surface enhancer. The membrane solution consists of 4.45 wt% Tecoflex EG-80A (Thermedics Inc.) and 0.85 wt% polydimethylsiloxane (DC360, 12500 cSt, Dow Corning) dissolved

in tetrahydrofuran. The membrane is applied by a single vertical dip resulting in a membrane with a thickness of 10 μm .

Example 4

This example illustrates how a membrane containing two types of polyurethane and one type of silicone can be constructed.

1.82 g Tecoflex EG-80A and 1.82 g Tecophilic HP-60D-20 (both polyurethanes purchased from Thermedics Inc.) are solvated in 800 ml

9.75THF:0.25H₂O. This solution is stirred for at least 60 hours. Then 128.41 g polydimethylsiloxane solution (prepared as described in example 1) is added to the solution thereby giving 18.65% siloxane of the total dry weight of polymer. A two-electrode system, where the working electrode, pre-coated with a layer of glucose oxidase which is approximately 4 μm thick, is spray-coated with the solution described in this example to achieve a membrane of this invention having a thickness of approximately 20 μm .

Example 5

This example illustrates how a membrane containing two layers of two kinds of polyurethane each containing polydimethylsiloxane can be constructed.

3.64 g Tecoflex EG-80A is solvated in 800 ml THF and to this is added 128.41 g polydimethylsiloxane solution as prepared in example 1. A two-electrode system, where the working electrode is coated with a layer of glucose oxidase is spray-coated with the solution described in the above to achieve a membrane layer, where the Tecoflex layer is in the range from 1 to 5 μm thick. Then subsequently the solution described in example 1 is sprayed on top of this membrane with a thickness in the range from 1 to 25 μm thick in order to achieve a sensitivity above 0.4 nA/mM/mm².

Example 6

This example illustrates how to perform a tensile test of thin polymer films of this invention.

For this purpose a commercial polymer, Inspire™ 2301 (www.inspirecomponents.com) with a comparable thickness (30 µm) to the outer membranes of this invention, was utilised to demonstrate the method and intended as a point of reference. The polymer thin film was cut in pieces approximately 10 × 20 mm² in size and the thickness measured using a precision micrometer measuring tool with an accuracy of ±1 µm. The tensile test was performed on a Lloyds instruments tensile rig LR5K using a 20 Newton measuring head for the measurements since prior experiments have shown that the force necessary to reach the ultimate tensile strength for the used polyurethane-silicone films is in the range from 1 to 10 Newton. The stress was applied to the polymer film by an elongation of 30 mm/min until the film breaks. The stress, σ , is defined as the ratio of the force on the sample, P , and the original cross-sectional area, A_0 : $\sigma = P / A_0$, and the strain, ε , as the ratio of the change in length of the sample, Δl , to its original length l_0 : $\varepsilon = \Delta l / l_0$.

Fig. 12 shows the stress-strain curves obtained for a commercial polyurethane, Inspire™ 2301.

WHAT IS CLAIMED IS:

1. A membrane comprising a continuous phase of one polymer (or a mixture of miscible polymers) and discrete domains of a second high molecular weight polymer with high oxygen permeability (permeability towards oxygen), where the polymers in each phase are immiscible, and where the second high molecular weight polymer has a domain size in the range from about 20 μm to about 1 nm, preferably from about 10 μm to about 10 nm, more preferred from about 5 μm to about 50 nm, and, when said product is used as a dense or mostly dense outer membrane of a glucose oxidase based biosensor, it results in

- a) a signal-to-noise ratio larger than 3,
- b) sensitivity larger than 0.4 nA/mM/mm²,
- c) an extended linearity range for measurements of glucose,
- d) good chemical stability, and
- e) good mechanical stability

during in-vivo use for measurements of glucose using said glucose oxidase based biosensor as defined herein.

2. A membrane, according to claim 1, wherein one of the two polymers is a hydrophilic polymer.

3. A membrane, according to any one of the preceding claims, wherein one of the two polymers is a hydrophobic polymer.

4. A membrane, according to any one of the preceding claims, wherein any hydrophilic polymer present is a water swellable polyurethane or compositions of block copolymers of polyurethane, for example, polyurethane/-polyethylene oxide, polyurethane/polytetramethylene ether glycol, or polyurethane/polydimethylsiloxane.

5. A membrane, according to any one of the preceding claims, wherein the polyurethane-polysiloxane block copolymer is utilized as a compatibilizer/emulsifier for stabilisation of polysiloxane domains.

5 6. A membrane, according to any one of the preceding claims, wherein one of the immiscible polymers is a PU/PEO or PU/polytetramethylene glycol (designated PTMG) copolymer.

10 7. A membrane, according to any one of the preceding claims, wherein the hydrophilic polymer is preferably one from the family of aliphatic, polyether based polyurethanes named Tecophilic from Thermedics Inc., more preferably Tecophilic HP60D-20 from Thermedics Inc.

15 8. A membrane, according to any one of the preceding claims, wherein the hydrophobic polymer is polydimethylsiloxane, preferably DC360 (viscosity 12,500 cSt, Medical grade) from Dow Corning.

20 9. A membrane, according to the preceding claim, which is prepared from a hydrophilic polymer which swells from about 1% to about 50% of its dry resin weight, preferably from about 5 to about 40%, more preferred from about 10 to about 30%, most preferred about 20%.

25 10. A membrane, according to any one of the preceding claims, containing one or more hydrophilic polymers.

11. A membrane, according to any one of the preceding claims, wherein the molecular weight of any hydrophobic polymer is at least about 10 kDa, preferably at least about 20 kDa, more preferred at least about 30 kDa, most preferred about 42 kDa, and preferably not more than about 60 kDa.

30 12. A membrane, according to any one of the preceding claims, having a thickness in the range from about 1 μm to about 60 μm about, preferably

from about 5 μm to about 50 μm , more preferred from about 20 μm to about 40 μm , most preferred about 35 μm .

13. A membrane, according to any one of the preceding claims, having a volume in the range from about $0.01 \times 10^{-10} \text{ m}^3$ to about $250 \times 10^{-10} \text{ m}^3$, preferably from about $0.5 \times 10^{-10} \text{ m}^3$ to about $150 \times 10^{-10} \text{ m}^3$, more preferred from about 10^{-10} m^3 to about $50 \times 10^{-10} \text{ m}^3$, most preferred about $5 \times 10^{-10} \text{ m}^3$.

14. A membrane, according to any one of the preceding claims, having an ultimate tensile strength in wet condition as defined herein in the range from about 0.1 MPa to about 50 MPa, preferably in the range from about 1 MPa to about 40 MPa, more preferred in the range from about 2 MPa to about 30 MPa, most preferred about 8 MPa.

15. A membrane, according to any one of the preceding claims, wherein the ultimate elongation of the polymer is in the range from about 150% to about 800% of its original length, preferably from about 200% and about 700%, more preferably from about 250% to about 600%.

16. A membrane, according to any one of the preceding claims, having a sufficient biocompatibility in vivo as defined herein.

17. A membrane, according to any one of the preceding claims, wherein the hydrophilic water swellable material is a polyurethane.

18. A membrane, according to any one of the preceding claims, wherein the content of hydrophobic material is in the range from about 1 % to about 50 % (weight/weight) of the total weight, preferably from about 5 % to about 25 %, more preferred from about 8 % to about 20 %, most preferred about 18.6 %.

19. A membrane, according to any one of the preceding claims, which is prepared using a hydrophobic polymer having a permeability towards oxygen in the range from about 7×10^{-12} to about $7 \times 10^{-10} \text{ cm}^3 \times \text{cm}/(\text{cm}^2 \times \text{s} \times \text{Pa})$, preferably from about 1.4×10^{-11} to about $3.5 \times 10^{-10} \text{ cm}^3 \times \text{cm}/(\text{cm}^2 \times \text{s} \times \text{Pa})$, more preferred from about 2.3×10^{-11} to about $2.1 \times 10^{-10} \text{ cm}^3 \times \text{cm}/(\text{cm}^2 \times \text{s} \times \text{Pa})$, most preferred from about $7 \times 10^{-11} \text{ cm}^3 \times \text{cm}/(\text{cm}^2 \times \text{s} \times \text{Pa})$.
20. A membrane, according to any one of the preceding claims, which can be e-beam sterilized using a dose $2 \times 25 \text{ kGy}$ without changing the properties in an undesired way.
21. A membrane, according to any one of the preceding claims, which has a long term stability of at least 232 days after production.
22. A membrane which shows no phase transitions in the region from 6°C to 45°C .
23. A membrane, according to any one of the preceding claims, which is prepared from a hydrophobic material having a viscosity above about 5000 centistokes, preferably above about 10,000 centistokes, more preferably above about 12,000 centistokes.
24. An implantable biosensor for measuring the reaction of an analyte and oxygen, the working electrode of the sensor including a layer of enzyme, characterized in that said biosensor has an outer membrane consisting of a membrane according to any one of the preceding claims.
25. An implantable biosensor, according to the previous claim, characterized in that the analyte is glucose.
26. An implantable biosensor, according to any one of the previous biosensor claims, characterized in that the enzyme is glucose oxidase.

27. An implantable biosensor, according to any one of the previous biosensor claims, having a sufficient low start up time as defined herein.

28. An implantable biosensor, according to any one of the previous biosensor claims, having a stable biosensor response for 3 days when inserted in the subcutaneous tissue.

29. An implantable biosensor, according to any one of the previous biosensor claims, comprising a double layer membrane, where the innermost layer, which in an amperometric biosensor (see Fig. 1) will be closest to the electrode, and is made from PU/PDMS or PU and the outermost layer is made from PU-PEO, PU-PTMG, PU-PEO/PDMS, PU-PTMG/PDMS, PU/PU-PEO/PDMS, or PU/PU-PTMG/PDMS.

30. An implantable biosensor, according to any one of the previous biosensor claims, comprising a triple layer membrane, where the innermost layer consists of PU, PU-PEO, or PU-PTMG, the second layer of PU-PEO/PDMS, PU-PTMG/PDMS, PU/PU-PEO/PDMS, PU/PU-PTMG/PDMS, and the third layer of PU-PEO, or PU-PTMG.

31. A two or more phase biocompatible membrane consisting of a membrane according to any of the preceding product claims.

32. A method for placing a layer consisting of two immiscible polymers on a substrate, for example, for preparing a membrane according to any one of the preceding membrane claims, characterized in

a) using a solvent, preparing a solution or suspension containing one or more polymers,

b) applying a part of said polymer solution or suspension through a spray nozzle to said substrate in such a way that a certain amount of said solvent is evaporated before the polymer solution/suspension reaches the substrate,

- c) after the remaining part of the polymer solution or suspension has reached the substrate, allowing a substantial part of the remaining amount of solvent present in the polymer solution/suspension to evaporate,
- 5 d) applying a part of said polymer solution or suspension through a spray nozzle to said substrate in such a way that a certain amount of said solvent is evaporated before the polymer solution/suspension reaches the substrate,
- 10 e) after the remaining part of the polymer solution or suspension has reached the substrate, allowing a substantial part of the remaining amount of solvent present in the polymer solution/suspension to evaporate, and
- 15 f) repeatedly applying a solution or suspension as described in step b) to said substrate and allowing the solvent to evaporate as described in steps b) and c) for a total of at least about 30 steps, in this order.

33. Any novel feature or combination of features described herein.

1 / 12

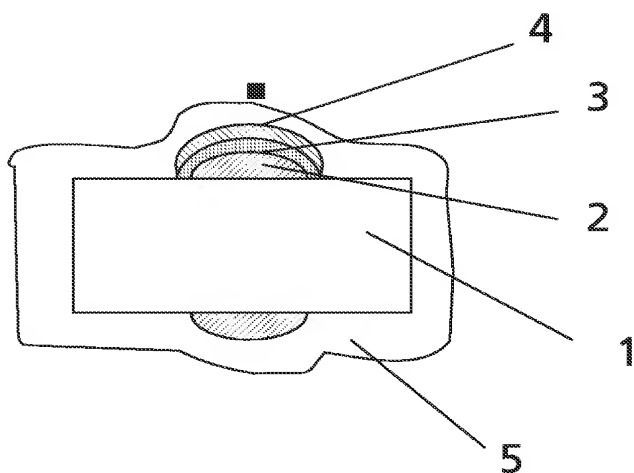


Fig. 1

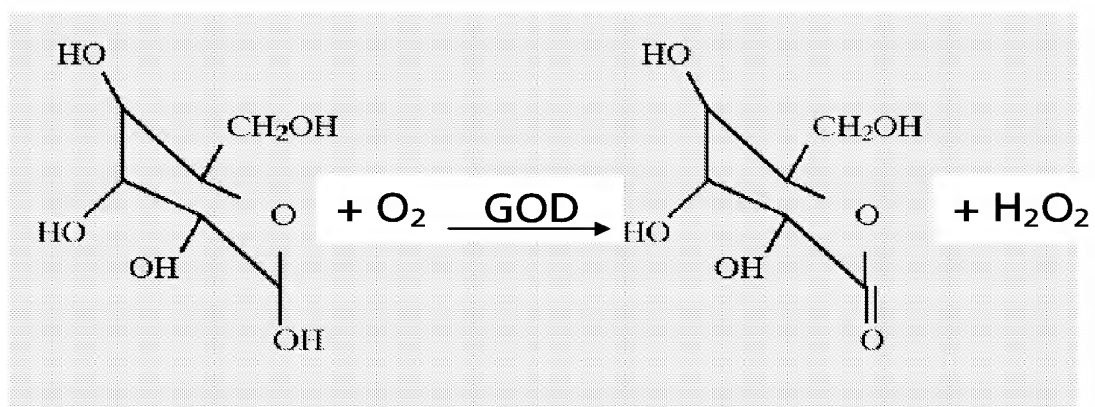


Fig. 2

2 / 12



Fig. 3

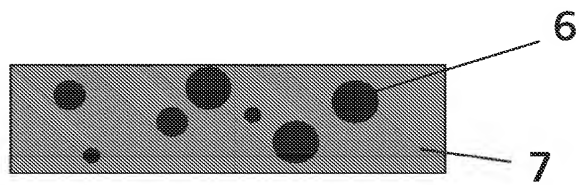


Fig. 4

3 / 12

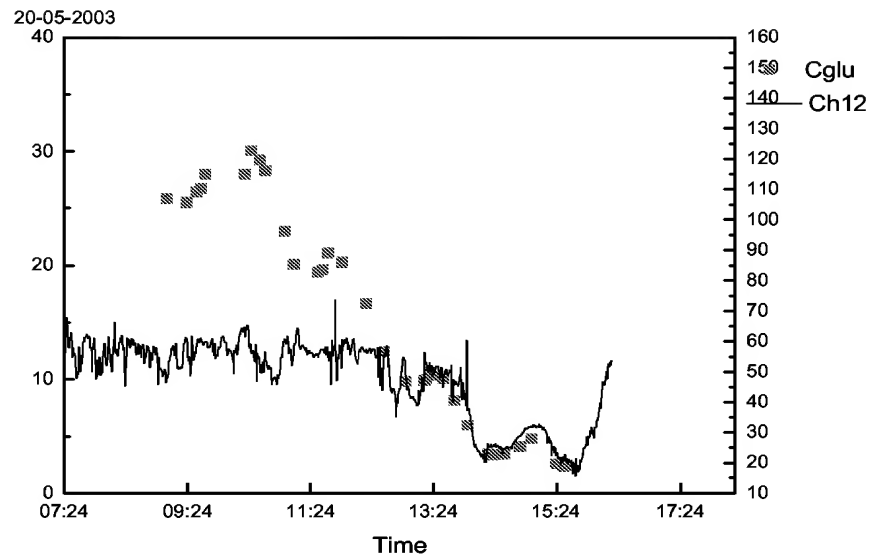


Fig. 5a

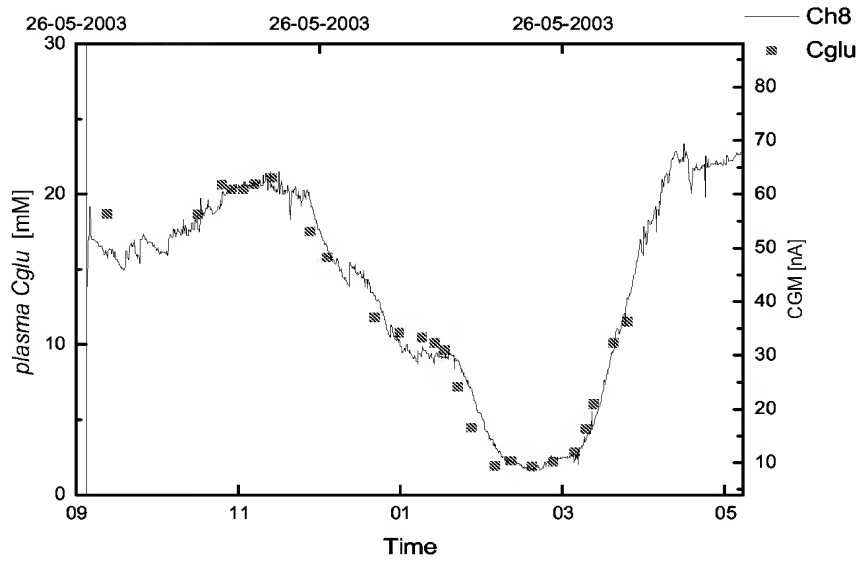


Fig. 5b

4 / 12

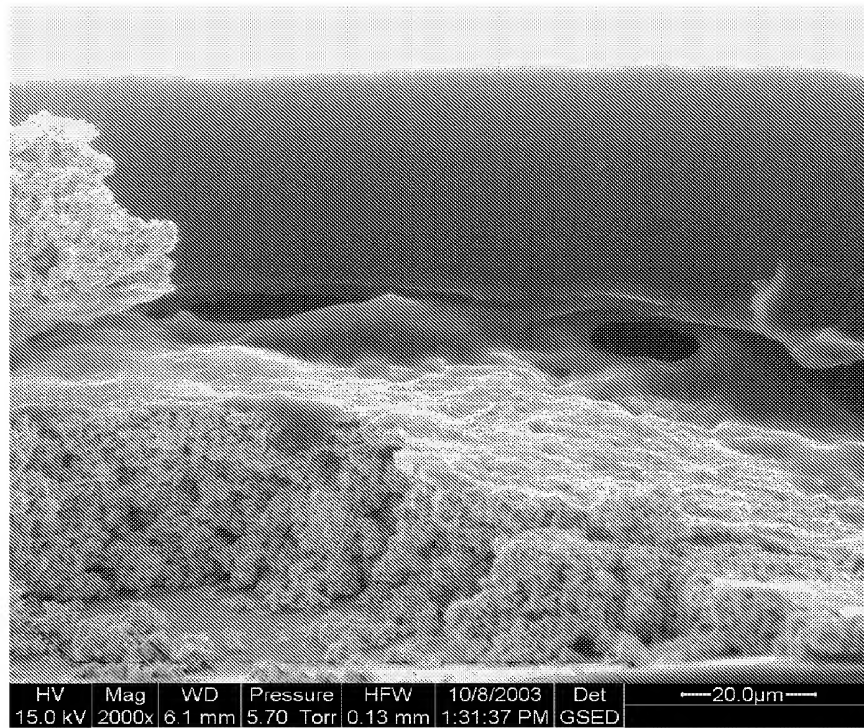


Fig. 6

5 / 12

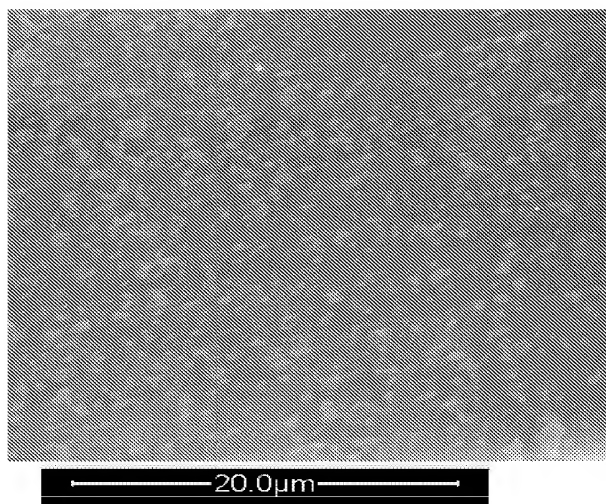


Fig. 7a

6 / 12

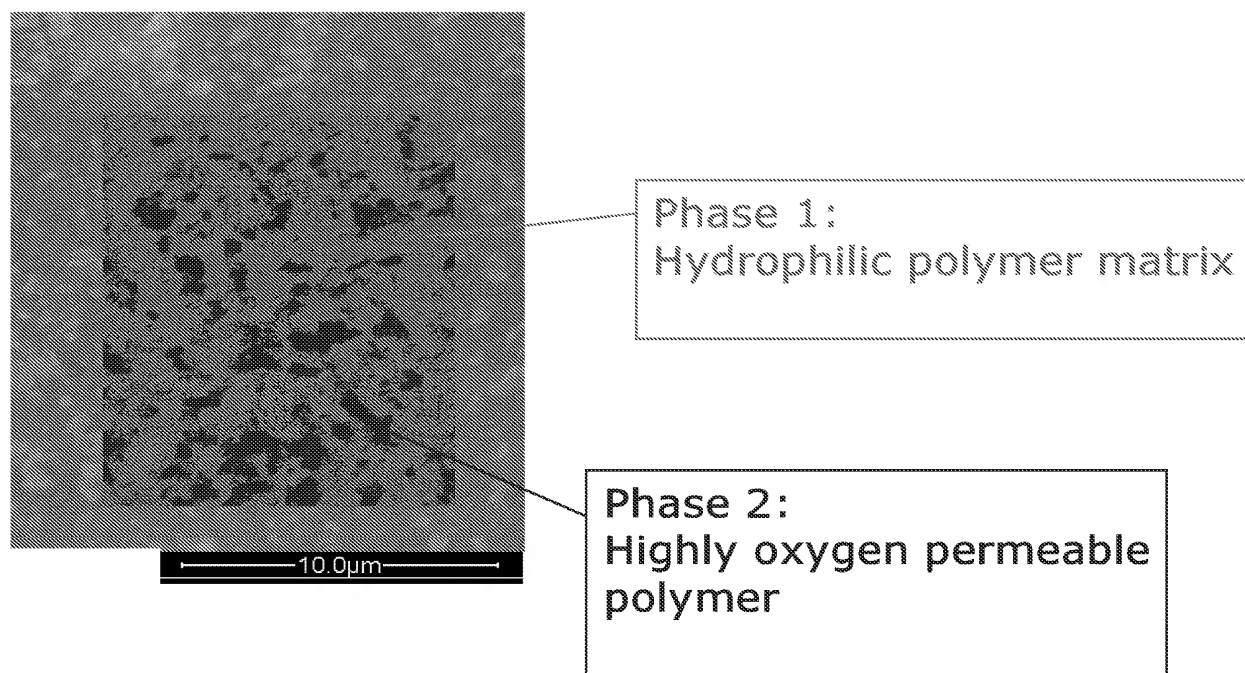


Fig. 7b

7 / 12

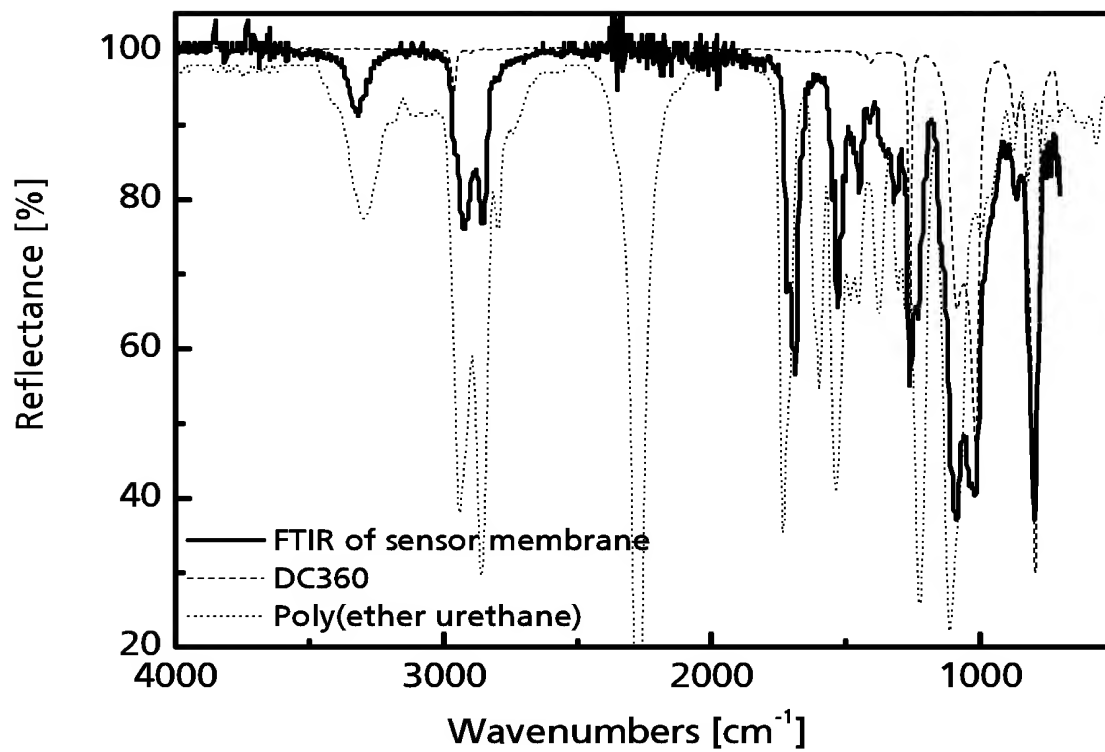


Fig. 8

8 / 12

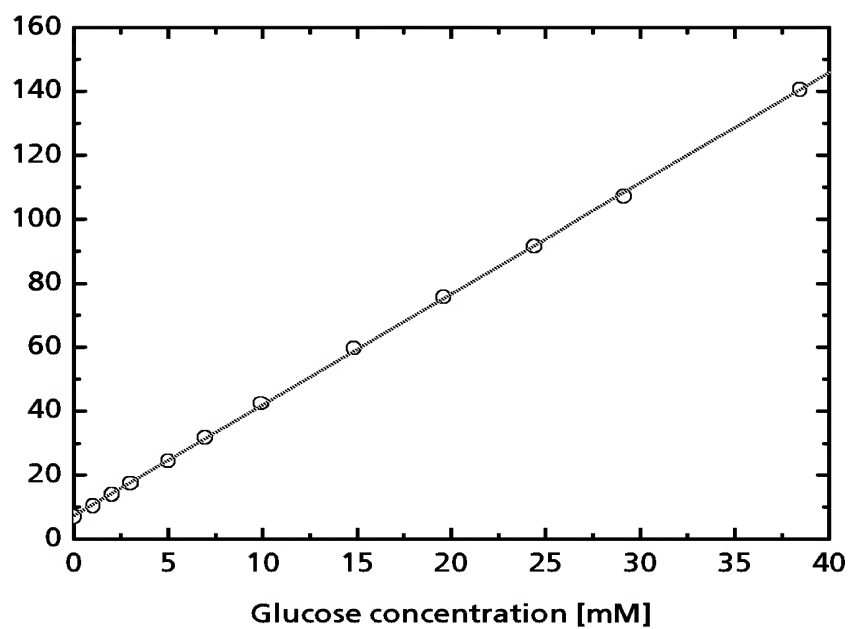


Fig. 9

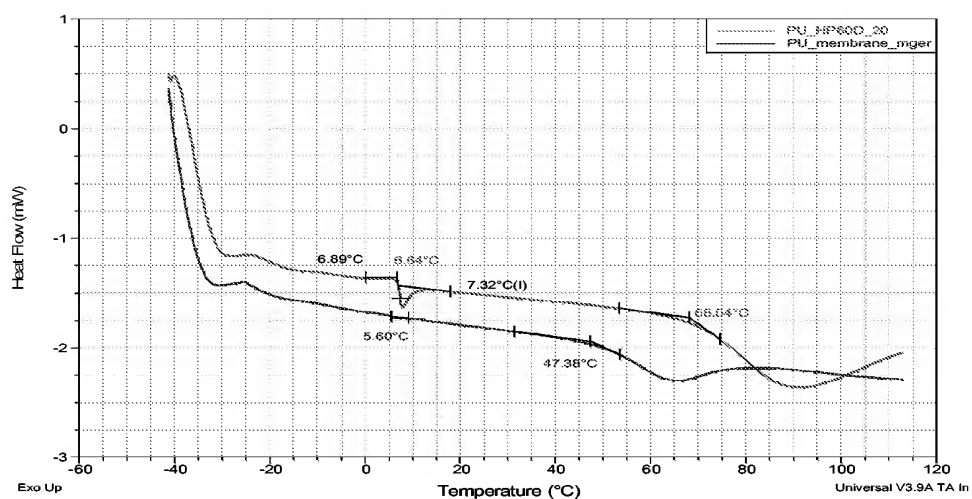


Fig. 10

9 / 12

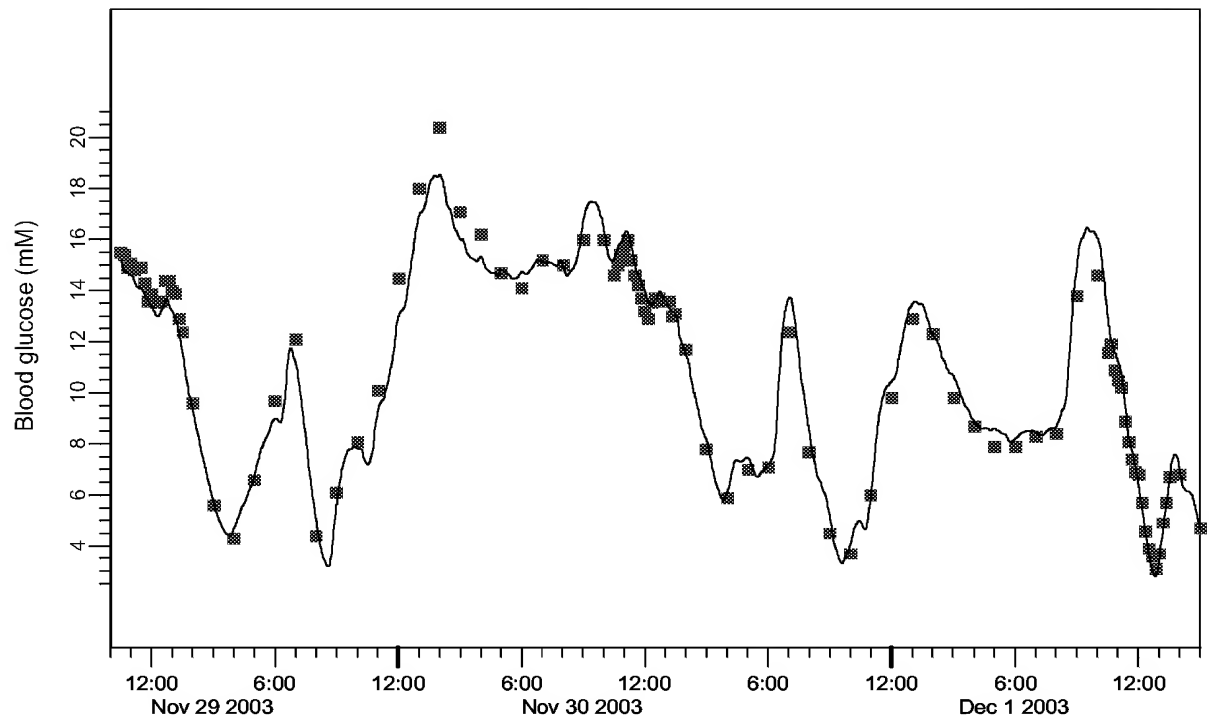


Fig. 11

10 / 12

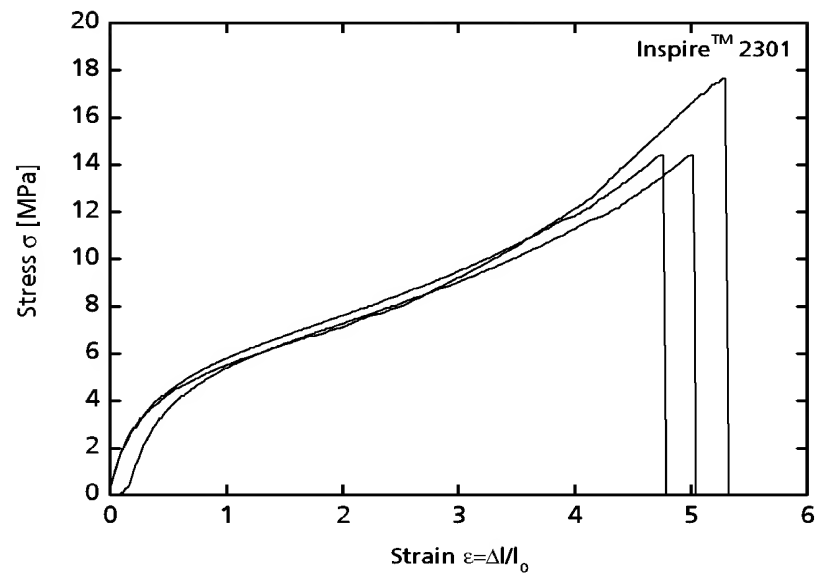


Fig. 12

11 / 12

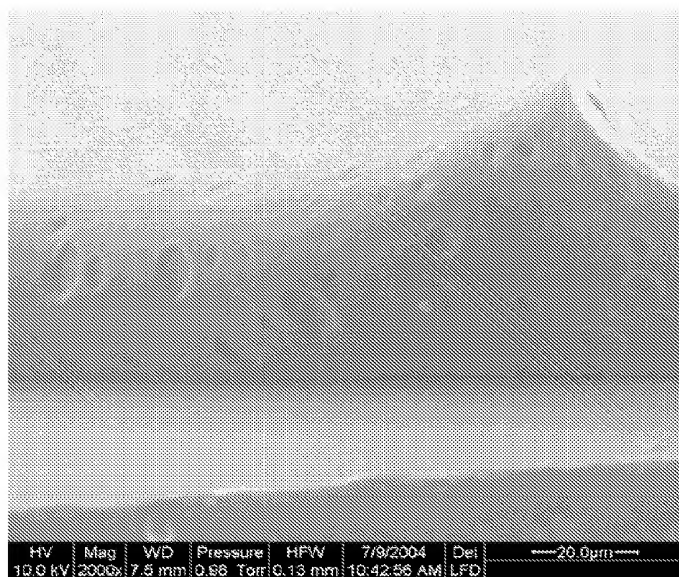


Fig. 13

12 / 12

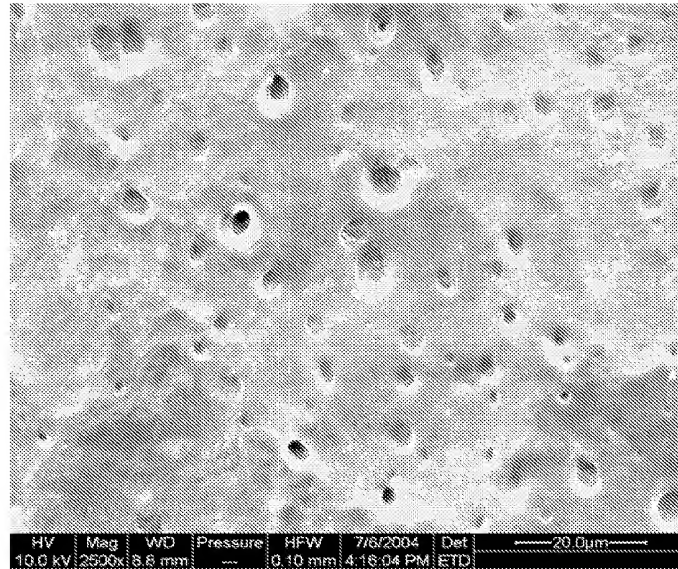


Fig. 14